REMARKS

Claims 1-2, 5-19, 25-33, 35-37 and 41 were pending in the subject application. By this amendment, Claim 17 has been canceled without prejudice or disclaimer; Claims 1-2, 5-6, 33, 35 and 41 have been amended; and new Claims 42-44 have been added. Applicants maintain that the amendments to the claims do not raise an issue of new matter. Support for the amendments can be found at least on page 6, paragraph [0026], page 13, paragraph [0054], page 14, paragraph [0059], Figure 5, and in Claim 17. The amendments place the application in condition for allowance or in better form for appeal. Accordingly, entry of the amendments is respectfully requested.

Rejections under 35 U.S.C. §112, First Paragraph

Claims 1-2, 5-19, 25-33, 35-37 and 41 are rejected under 35 U.S.C. §112, first paragraph, for failing to comply with the enablement requirement for the full breadth of the claims.

Applicants respectfully traverse this rejection.

In the previous reply, applicants addressed the Examiner's concerns regarding the 1954 publication of Mason et al., which the Examiner indicated is the closest prior art to the instantly claimed invention. The Examiner also indicated that Wilder et al. (1996), Erdi et al. (1996) and Chatel et al. (1992) raise concerns regarding the unpredictability of using any radiolabeled antibody to melanin for radioimmunotherapy and/or radio imaging and that as such it is the position of the Examiner that it would require undue experimentation for one of skill in the art to perform the method of the claims as previously written.

The claims have hereinabove been amended so that each independent claim requires that the radiolabeled antibody is a monoclonal antibody and that the radiolabeled monoclonal antibody specifically binds to melanin.

The Examiner's concerns with respect to the 1992 and 1996 articles by Wilder et al., Erdi et al. and Chatel et al. are addressed below. It is noted that these articles are

review articles directed to the general field of radioimmunotherapy and radioimaging and do not specifically address therapy and imaging of melanin-containing tumors.

The Examiner indicated that Wilder et al. (1996) disclose in their Abstract that challenges that currently face radioimmunotherapy include circulating free antigen, binding of antibodies to nonspecific Fc receptors, insufficient tumor penetration, antigenic heterogeneity and insufficient antigen expression, antigenic modulation, and development of human antimouse antibodies. However, applicants note that Wilder et al. also disclose several solutions to each of these concerns further on in their article, e.g. in Table 4 on page 1393. The following points are also relevant in regard to the presently claimed invention. In regard to circulating free antigen, the greatest concentration of free melanin is expected to be in the tumor and its immediate vicinity from dead or dying tumor cells; therefore, this issue should not raise a problem for imaging or treating the tumor. In contrast to conventional tumor antigens, melanin is insoluble. If melanin particles separate from the tumor, they are expected to be sequestered by macrophages in the liver and spleen, thus becoming intracellular and not accessible to antibodies that cannot be internalized by the cell. In regard to tumor penetration by the antibody, antigenic heterogeneity, insufficient antigen expression, and antigenic modulation, it is noted that the antibody does not have to penetrate the tumor cells to be effective for therapy or imaging since the antibody can bind to melanin from dead or dying tumor cells and/or the antibody can gain access to melanin in the cells following disruption of the cell membrane. In regard to therapy, adjacent tumor cells can be killed by the radiation even if the tumor cells do not contain melanin (see, e.g., Figure 10 of application). As tumor cells are killed by therapy, more melanin would be expected to accumulate in the tumor. Thus, efficacy of treatment may increase with subsequent treatment cycles. Further, as noted in Wilder et al. (Figure 2), antibodies can be "humanized" to prevent possible development of human antimouse antibodies. In this regard, it is noted that the U.S. Food and Drug Administration (FDA) has approved several humanized monoclonal antibodies for cancer treatment in human subjects,

including, for example, Herceptin® for HER2-positive metastatic breast cancer, Erbitux® for advanced colorectal cancer that has spread to other parts of the body, Rituxan® for Non-Hodgkin's Lymphoma (NHL), Mylotarg® for a form of bone marrow cancer (CD33 positive acute myeloid leukemia), Campath® for B-cell chronic lymphocytic leukemia, and Avastin® for inhibiting angiogenesis.

The Examiner further indicated that Wilder et al. also teach that the transport of antibodies through the interstitial space of a tumor is impeded by antigen binding and relatively large extravascular spaces (Wilder et al., page 1387). As noted above, in the present invention, the antibody does not have to penetrate tumor cells to be effective since the antibody binds to melanin from dead or dying tumor cells and since adjacent tumor cells can be killed by the radiation.

The Examiner also indicated that Erdi et al. (1996) teach that there are problems which limit the use of radioimmunotherapy including low uptake of radiolabeled antibody, low target:non-target ratios and inhomogeneous distribution of antibody within the tumor (Erdi et al., page 2009) and that the same problems are found with radioimaging (see Chatel et al. (1992)). In reply, as noted above, in the present invention, the antibody does not have to be taken up by the tumor cell to be effective for therapy or imaging since the antibody can bind to melanin from dead or dying tumor cells and/or the antibody can gain access to melanin in the cells following disruption of the cell membrane. The concentration of released melanin is expected to be highest in the tumor and its immediate vicinity. If melanin particles separate from the tumor, they are expected to be sequestered by macrophages in the liver and spleen, thus becoming intracellular and not accessible to antibodies that cannot be internalized by the cell. The impact of inhomogeneous distribution of antibody within the tumor is reduced since the radiation kills adjacent tumor cells.

Applicants note that since the publication of the 1954 research report by Mason et al. and the 1992 (Chatel et al.) and 1996 (Wilder et al.; Erdi et al.) review articles cited by the Examiner, radioimmunotherapy has advanced by a quantum leap as evidenced,

for example, by the attached 2004 review article by Milenic et al. (Antibody-targeted radiation cancer therapy, Nature Rev. Drug Discovery 3: 488-498, 2004). Two radionuclide monoclonal antibody therapies have been approved by the U.S. FDA and several more are in clinical trials (see, e.g. Milenic et al., Abstract and Table 1, page 490). Zevalin® and Bexxar® were approved by the FDA in 2002 and 2003, respectively, and are currently very successfully used in treatment of refractory and recurrent non-Hodgkin's lymphoma (NHL). In their conclusions (page 496), Milenic et al. state "[a]fter more than two decades, mAb-targeted therapies are generally recognized as making a significant impact on cancer therapy."

The present invention represents a major advance in the treatment of melanoma. The last drug to treat melanoma was approved by the FDA 30 years ago despite the fact that according to the American Cancer Society, melanoma accounts for about 75% of all skin cancer related deaths. Even worse, for patients with metastatic melanoma, the 5-year survival rate is only about 5%. The present invention targets melanin-containing tumors with specificity and delivers an effective dose of radiation that is not subject to drug resistance. As a testimonial to the importance of the research underlying the present invention, the work was published in the highly acclaimed, peer-reviewed journal, the Proceedings of The National Academy of Sciences (Dadachova et al. PNAS 101(41): 14865-70, 2004, copy submitted with previous reply).

Finally, applicants note that even if the invention as claimed did read on an inoperative embodiment, "[t]he presence of inoperative embodiments within the scope of a claim does not necessarily render a claim nonenabled. The standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984)" (MPEP §2164.08(b)).

Applicants respectfully maintain that the specification is enabling for the skilled artisan to practice the claimed invention without undue experimentation. In view of the

amendments and remarks made hereinabove, and in view of the remarks regarding Mason et al. (1954) made in applicants' previous reply, reconsideration and withdrawal of this ground of rejection are respectfully requested.

CONCLUSIONS

In view of the remarks and amendment made hereinabove, reconsideration and withdrawal of the rejections in the September 14, 2006 Final Office Action and passage of the pending claims to allowance are respectfully requested. If there are any minor matters preventing the allowance of the subject application, the Examiner is requested to telephone the undersigned attorney.

No fee is deemed necessary in connection with the filing of this reply. However, if any fee is required with this submission or to preserve the pendency of the subject application, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 01-1785.

Respectfully submitted,

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Dated: December 12, 2006
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By _____
Alan D. Miller, Reg. No. 42,889

ANTIBODY-TARGETED RADIATION CANCER THERAPY

Diane E. Milenic, Erik D. Brady and Martin W. Brechbiel

Several monoclonal antibodies are now approved for cancer therapy, such as rituximab, an anti-CD20 monoclonal antibody for the treatment of B-cell non-Hodgkin's lymphoma. Such 'naked' antibodies can recruit the body's immune effector mechanisms to kill cells expressing the target of the antibody. In recent years, the linking of radionuclides to antibodies to either augment inherent activity or to exploit the specific targeting properties of monoclonal antibodies has been a major area of development. Two radionuclide-bearing monoclonal antibody therapies have recently been approved by the US FDA, and several more are in clinical trials. Here, we discuss the development and use of radiolabelled monoclonal antibody therapies, with a focus on radiolabelled monoclonal antibodies that have been evaluated in clinical trials.

The efficacy of traditional cytotoxic cancer therapies generally comes with the price of significant toxicity to normal cells, which can limit the success of therapy. But in the past two decades, greater understanding of the molecular differences between cancer cells and normal cells has led to the development of therapies that target cancer cells, including antibodies directed at tumour-associated antigens. The targeted nature of such therapies offers the promise of greater efficacy and less toxicity, and potentially greater treatment success.

A key step in the emergence of antibody-targeted cancer therapy was the development of hybridoma/monoclonal antibody (mAb) technology by Kohler and Milstein, whose seminal publication in 1975 described the fusion of a plasma cytoma with spleen cells and subsequent isolation of hybrids that secreted antibody with a predefined specificity[1]. This breakthrough resurrected the concept put forth by Ehrlich a century ago that antibodies might serve as 'magic bullets'[2].

During the 1980s, the generation of murine mAbs against tumour-associated antigens became a focal point of research. Multiple preclinical studies provided proof-of-concept for the potential of mAbs in therapeutic applications for cancer treatment. Preclinical and clinical investigations with murine mAbs also highlighted several issues that required attention before success could be achieved in cancer management. Foremost of these was the seemingly inevitable production of human anti-murine immunoglobulin antibodies (HAMA) after one to three treatments in immunocompetent patients[3]. Other factors limiting treatment included inadequate therapeutic dose delivered to tumour lesions; insufficient activation of effector function(s); slow blood compartment clearance; low mAb affinity and avidity; transport to, or targeting of, normal organs; heterogeneous antigen distribution on tumour cells; and insufficient tumour penetration[3]. Some of these limitations were addressed by chemical modification of the mAb, but most of these challenges have been addressed with genetic engineering techniques[4]. This effort has primarily been applied to eliminating HAMA through the production of chimeric mAbs, grafting of complementarity-determining region (CDR) or complete humanization of the protein[4]. Even so, some of these obstacles — such as tumour heterogeniety and penetration — are unresolved and research continues in these areas.

In 1997, rituximab (Rituxan; Genentech/Biogen Idec) became the first antibody to be approved by the FDA for cancer therapy. Rituximab, which is an anti-CD20 antibody for the treatment of non-Hodgkin's lymphoma (NHL), has been followed by several other antibody-based cancer therapies, such as trastuzumab (Herceptin; Genentech/Roche), an anti-HER2 antibody for the treatment of HER2-receptor-positive breast cancer.

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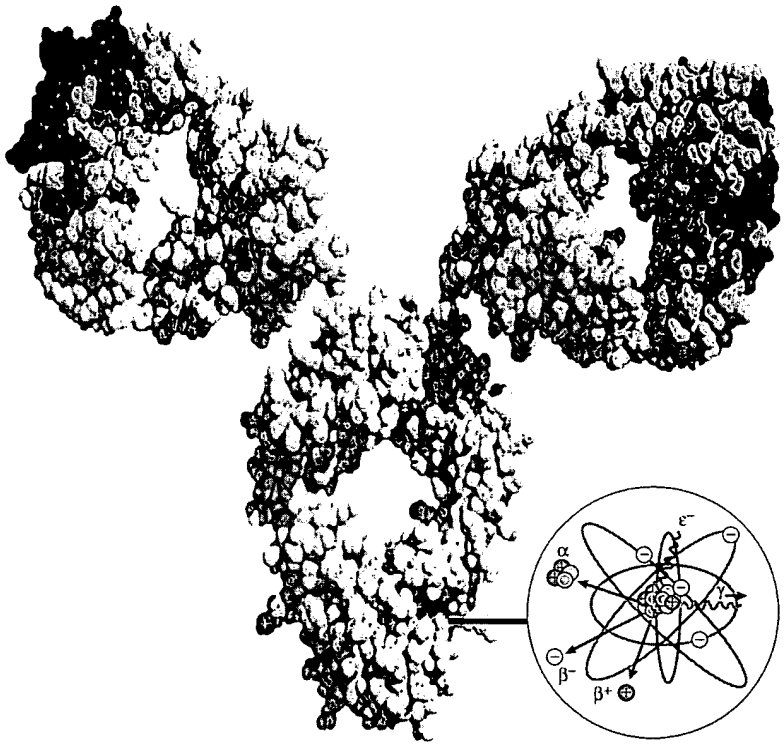

Figure 1 | **A monoclonal antibody linked to a radionuclide.** Monoclonal antibodies are linked to various radionuclides through several methods that are primarily based on the chemical characteristics of the specific radionuclide. Halogens, such as ^{131}I, are routinely introduced by direct halogenation of tyrosine residues of the protein. Metallic radionuclides, such as ^{111}In or ^{90}Y, require chelation of the metal through a suitable ligand. This chelating agent is routinely introduced through a reactive functional group that targets *N*-terminal and ε-amines of lysine residues. Such linking moieties include isothiocyanates, bromoacetamides, maleimides (post-thiolation of the protein) and active esters. Variations of these linking chemistries with proteins are also used for the indirect introduction of radio-halogens, including, but not limited to, ^{211}At.

Although such antibody therapies have shown significant success in cancer treatment, strategies to increase their efficacy are urgently needed.

One such strategy is to link antibodies against tumour-associated antigens to highly toxic radioisotopes, which brings to bear the killing power of these isotopes on tumour cells. Recent advances have brought us to the stage where investigators are finally able to fully explore the real therapeutic potential of radiolabelled mAbs (FIG. 1). With the elimination of many obstacles and a better understanding of the inherent limitations of mAbs, coupled with interest and support from industry, several radiolabelled mAbs have been, or are now being evaluated in Phase III clinical trials (TABLE 1). In the past two years, the US FDA has approved the use of two anti-CD20 mAb regimens involving radionuclides for the treatment of NHL: Zevalin (Biogen Idec) and Bexxar (Corixa/ GlaxoSmithKline), which are based on the radiolabelled mAbs ^{90}Y ibritumomab and ^{131}I tositumomab, respectively, making further targeted radiation therapy products probable[5]. This review describes radiolabelled-mAb-directed approaches, with an emphasis on the components (protein, radionuclide and chemistry), and discusses clinical trials of radiolabelled mAbs in haematological cancers.

The radionuclide

Choosing the most appropriate radionuclide for treatment on the basis of the size and presentation of the disease is crucial. Although this seems obvious, a survey of the literature indicates that this paradigm has not been broadly observed. As fractionation of both chemotherapeutics and external beam radiation are routine regimens, it is curious that treatment with a single dose containing the 'best radioisotope' has become such an undue focus. No single radionuclide is likely to address every therapeutic need, as disease does not exclusively present in a sole form, particularly in Phase I/II trials. Unfortunately, isotope selection is also often driven by economic rather than medical or scientific considerations, which could negatively affect both pre-clinical and clinical trials. Ultimately, the limitations of the targeting vehicles and radionuclides will be more clearly defined and a rational plan for future clinical trials will follow.

Some of the crucial considerations for successful targeted radiation are those variables pertaining to the radiation; that is, emission type, energy/range of emission and the half-life. A sampling of available isotopes (TABLE 2) results in several alternatives comprising three types of emission: β^--particles, α-particles and Auger electrons[5] (FIG. 2). Historically, β^--emitters have received the greatest focus. The emission path lengths of β^--emitters are relatively long, yet sparse (mean range of 275 μm, maximum range of 500–600 μm for ^{90}Y), with low linear energy transfer (LET) (FIG. 3). Energy deposition takes place at some distance from the actual decay event[6]. Therapeutic benefit results from 'crossfire'; that is, the cell targeted with the radionuclide — which can be targeted to the cell surface or designed to be internalized within the cell — is not necessarily the effective target of the decay event. Given this, some of the limitations of β^--emitters become clearly evident: treatment of single-cell metastatic diseases, leukaemias and disseminated diseases cannot be adequately addressed with β^--emitters[6].

Advantages of β^--emitters include their ability to bypass tumour antigen heterogeneity and differential penetration of the mAb. The capacity to uniformly target the entire lesion becomes possible when the emission range exceeds the radius of the targeted lesion[7]. Convenience, availability and familiarity with radiolabelling chemistry has traditionally supported the use of the iodine isotopes — for example, ^{131}I. Other clinically relevant β^--emitters include ^{90}Y, ^{67}Cu, ^{186}Re and, more recently, ^{177}Lu, with others having either been investigated or proposed (see below). Discussion of these will be limited here, except to state that emission energy and half-life requirements can be met with a small cross-section of isotopes that are already available[5]. Direct radio-iodination occurring with extant tyrosine moieties of the protein has dominated this field. The convenience of this method, however, is compromised by potentially rapid de-iodination of the protein post-internalization, a characteristic that is lacking when radiometals are used[8,9]. ^{90}Y has a pure β^--emission that delivers ~4.5 times more radiation per mCi to a tumour than does ^{131}I. The greater emission range of ^{90}Y means that most of the decay energy is deposited in tumours

Table 1 | **Representative monoclonal antibodies in advanced radioimmunotherapy clinical trials**

Antibody	Antibody form	Radionuclide	Antigen	Disease	Clinical trial status
Tositumomab (Bexxar)	muIgG2a	^{131}I	CD20	NHL	Approved by FDA
Epratuzumab (Lymphocide)	huIgG1 (LL2)	^{90}Y	CD22	NHL	Phase III
Labetuzumab (CEA-Cide)	huIgG1	^{90}Y	CEA	Colorectal, breast, lung, pancreatic, stomach carcinoma	Pending Phase III
chTNT-1/B (Cotara)	chIgG1	^{131}I	DNA	Glioblastoma multiforme, anaplastic astrocytoma	Phase III
^{131}I Lym-1 (Oncolym)	huIgG1	^{131}I	HLA-DR10	NHL, CLL	Phase II/III
Pemtumomab (Theragyn)	muIgG1	^{90}Y	PEM	Ovarian, gastric carcinoma	Phase III
Ibritumomab tiuxetan (Zevalin)	muIgG1	^{90}Y	CD20	NHL	Approved by FDA; Phase IV

CEA, carcinoembryonic antigen; CLL, chronic lymphocytic leukaemia; hulg, human immunoglobulin; mulg, murine immunoglobulin; NHL, non-Hodgkin's lymphoma; PEM, polymorphic epithelial mucin.

only if their diameter is 1 cm or more. Unlike ^{131}I, ^{90}Y lacks an imageable emission, thereby requiring dosimetry using ^{111}In for γ-SCINTIGRAPHY and SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY IMAGING; and although similar, these metals have different chemistries, and so such an approach might not be entirely accurate. Furthermore, the longer emission range that is associated with ^{90}Y might be disadvantageous, in that irradiation of normal tissue surrounding the lesion might result. In general, the longer-range β^--emissions that occur during circulation and subsequent myelosuppression is a consistent limitation[10]; exceptions might be realized with ^{177}Lu and ^{67}Cu, owing to their decreased β^--emission energy and range. Both have been evaluated in clinical trials for therapeutic efficacy[11–13], and possess imageable γ-emissions that allow determination of disease extent, calculation/prediction of dosimetry and monitoring of therapeutic efficacy. It should be noted that this same γ-emission contributes to both efficacy and normal tissue toxicity, illustrating the choices and compromises that must be balanced in radionuclide selection. Despite these concerns and the limitations of β^--emitters, their advantages mean that their use continues to dominate preclinical and clinical trials, and indeed both of the radiolabelled mAbs approved by the FDA are armed with β^--emitters.

The list of α-emitting radionuclides qualified for targeted radiation therapy is admittedly short, largely owing to half-life constraints. At present, only ^{212}Bi, ^{213}Bi and ^{211}At are being actively studied[14,15]; additionally, ^{225}Ac (TABLE 2) has shown promise despite concerns about the lengthy half-life and trafficking of decay products *in vivo*[16]. The bismuth radioisotopes ^{212}Bi and ^{213}Bi are available from generators based on ^{224}Ra and ^{225}Ac, respectively, and decay via branched pathways that result in both α- and β^--emissions. ^{212}Bi possesses a high-energy γ-emission in 32% abundance that ^{213}Bi lacks, and so the latter is generally considered a more attractive candidate for radioimmunotherapy[17]. Protocols that are analogous to radio-iodination chemistry were initially applied for ^{211}At; however, this has been supplanted with linking reagents that address the inherent instability of direct tyrosine protein labelling with this isotope (see below)[18].

As a group, the α-emitters produce high-energy particles (4–9 MeV) that travel relatively short distances (40–100 μm). They are characterized by dense emission path lengths of high LET, ~400 times greater than that of β^--emitters (80 versus 0.2 keV per μm), with energy deposition taking place immediately at the decay site[6] (FIG. 2). The emission of α-particles is highly cytotoxic at a dose rate of 1 cGy per hr[19]. The short range limits their use to a complementary scale of disease. Most, if not all, of the therapy results from the direct emission of the α-particle, making the cell targeted with the radionuclide and the immediate neighbouring cells the effective targets. In contrast to β^--emitters, a very low number of nuclear traversals (one to three) are generally all that is required to kill a cell with an α-emitter[6]. An inherent limitation of these α-emitters is their relatively short physical half-life. This, coupled with their emission path length, has generally been thought to limit the use of α-emitters to leukaemias, highly vascularized tumours and metastatic/disseminated disease in which adequate access, targeting time and appropriate disease size converge. In addition, bone-marrow purging for transplant conditioning, as well as selective tumour vasculature targeting with the goal of tumour eradication, seem obvious avenues for future work[20,21]. Adjuvant therapies might also be viable[15,22].

Auger emitters, such as ^{67}Ga, ^{195m}Pt, ^{123}I and ^{125}I (TABLE 2), have received the least attention. This is due to the accepted premise that their extreme cytoxicity, and therefore efficacy, is limited by the prerequisite for emissions to occur within the cell nucleus[23]. Despite this apparent limitation, studies have demonstrated that Auger emitters might have a significant role as therapeutics, even if their clinical use might be limited to irradication of microscopic residual disease[24,25].

SCINTIGRAPHY
Imaging of γ-emissions.

SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY IMAGING
An imaging technique using corrected photon emissions.

Table 2 | **Therapeutic radionuclides**

Radionuclide	Type	Half-life	E_{max} (MeV)	Mean range (mm)	Imageable
^{90}Y	β	2.7 d	2.3	2.76	No
^{131}I	β, γ	8.0 d	0.81	0.40	Yes
^{177}Lu	β, γ	6.7 d	0.50	0.28	Yes
^{153}Sm	β, γ	2.0 d	0.80	0.53	Yes
^{186}Re	β, γ	3.8 d	1.1	0.92	Yes
^{188}Re	β, γ	17.0 h	2.1	2.43	Yes
^{67}Cu	β, γ	2.6 d	0.57	0.6	Yes
^{225}Ac	α, β	10 d	5.83	0.04–0.1	Yes
^{213}Bi	α	45.7 min	5.87	0.04–0.1	Yes
^{212}Bi	α	1.0 h	6.09	0.04–0.1	Yes
^{211}At	α	7.2 h	5.87	0.04–0.1	Yes
^{212}Pb	β	10.6 h	0.57	0.6	Yes
^{125}I	Auger	60.1 d	0.35	0.001–0.02	Yes
^{123}I	Auger	13.2 h	0.16	0.001–0.02	No
^{67}Ga	Auger, β, γ	3.3 d	0.18	0.001–0.02	Yes
^{195m}Pt	Auger	4.0 d	0.13	0.001–0.02	No

Linking radionuclides to protein

This aspect of radioimmunotherapy has been one of considerable activity, as researchers have sought to balance the conditions required to achieve a radiolabelled product with adequate stability of the resulting complex, within the constraints imposed by isotope chemistry and half-life. As should be expected, the choices of realistic isotopes and chelating agents have narrowed and focused as this field has matured.

Direct radio-iodination (with ^{131}I, ^{125}I or ^{123}I) is well established and will not be addressed here. All metallic radionuclides require chelation chemistry for attachment to a mAb (FIG. 4). Bifunctional chelating agents (BCAs) — CHELATES that possess specific functional groups that allow both conjugation to proteins and stable complex formation with metallic radionuclides — are required, and this is an active area of research[26]. Similarly, ^{211}At also requires a linking agent, as direct radio-halogenation is inappropriate. Development of BCAs exceeds the scope of this work and only a brief discussion follows.

The suitable radiometals are diverse in their properties and coordination chemistry, so, unfortunately, no single BCA is suitable for all of these metals[26]. A selection of examples is provided in FIG. 5. The ultimate goal of 'instant' radionuclide complex formation with infinite stability, or zero dissociation, although laudable, has proven non-trivial. Numerous chemical criteria must be considered in the choice of chelating agent; for example, its design and its actual use. Characteristics of the metal, such as COORDINATION NUMBER, ionic radius, METAL-BINDING CHARACTER (hard versus soft) and reactivity (hydrolysis versus complexation) must also be considered with respect to chelate design[26]. A BCA might form and maintain an adequately stable metal complex, but the formation kinetics might also render the BCA impractical for the intended radionuclide.

For example, DOTA (1,4,7,10-tetra-azacylcododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) forms highly stable and kinetically inert complexes with ^{212}Bi and ^{213}Bi (REF. 25). However, Bi(III) complexation kinetics with DOTA require 15–45 minutes for reaction completion. The half-lives of the radionuclides are 60 and 46 min, respectively, making this particular combination wasteful[27]. Higher temperatures traverse this in part, but are limited owing to the inherent characteristics of the protein targeting vehicle. In contrast to macrocyclic BCAs, acyclic BCAs tend to show far faster complex-formation rates, but these are not as stable, representing another forced compromise. The acyclic CHX-A″, a cyclohexyl-DTPA (diethylenetriamine pentaacetic acid) (FIG. 5), has been shown to be a viable alternative to DOTA for the labelling of mAbs with Bi(III) isotopes[28,29]. This BCA complexes with bismuth 'instantaneously' ($t_{1/2}$ = 0.27 s) and is sufficiently stable for clinical trials[30]. In addition, it was reported to have similar stability with the β^--emitter ^{177}Lu versus DOTA and PA-DOTA, the latter of which is used in clinical trials in combination with ^{177}Lu (REFS 11,12,31). In summary, this ligand not only provides considerable versatility for radiolabelling mAbs with the α-emitters ^{213}Bi and ^{212}Bi, but also with β^--emitters such as ^{90}Y and ^{177}Lu, allowing a wider range of clinical applications[29,32,33]. ^{67}Cu remains an interesting candidate for therapy with regards to emission energy, half-life and imageable emissions; as such, development of the chemistry remains active[34]. The choice of BCA for ^{67}Cu remains an open and unresolved topic; several different macrocyclic chelating agents have been touted as stable and inert with ^{67}Cu, despite reports of trans-chelation to superoxide dismutase and detection in patients' ceruloplasmin[35–37]. Production and availability questions currently compromise ^{67}Cu, and so ^{64}Cu might eventually be judged to be more viable[38].

The chemistry for linking ^{211}At to proteins has been reviewed and has been dominated by aryl active ester reagents that have advanced to clinical trials[18,39]. Issues of inadequate *in vivo* stability for general application in clinical settings are unresolved, which might be addressed with a better understanding of the chemistry of the element itself.

The protein

Although only present at a low concentration in most targeted radiation therapies, the protein and its contribution to therapeutic action should not be discounted. Direct tumour-cell killing can be achieved by two separate pathways: antibody-dependent cell cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC)[40]. ADCC is triggered when the Fcγ receptor on effector cells engage the Fc region of an antibody bound to a tumour cell. CDC is induced when complement component C1q binds to the Fc region of antibody bound to the tumour cell surface. In either situation, cell killing can proceed through a cell-dependent (phagocytosis) or a cell-independent mechanism (lysis). The Fc portion of the mAb can also be engineered to enhance CDC activity. Cell killing by apoptosis has also been attributed to binding CD20 as applied to B lymphocytes[41].

CHELATES
A ligand–metal complex.

COORDINATION NUMBER
The number of metal acceptor sites occupied by ligand donors.

METAL-BINDING CHARACTER
Acids (metals) and bases (ligands) can be categorized on the basis of polarizability: 'hard' (less polarizable) metals tend to form stronger complexes with 'hard' ligands than with 'soft' ligands; the converse is also applicable.

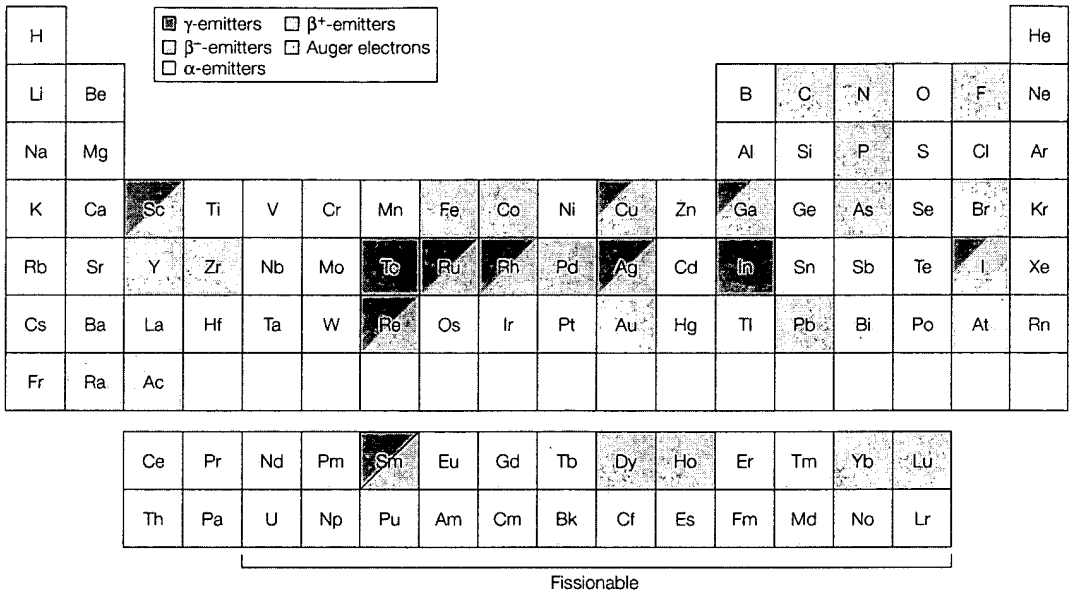

Figure 2 | **A representation of the periodic table, highlighting those elements that are of interest for nuclear medicine and radiation oncology applications.** The elements are colour coded by emission type. With the exception of the halogens, most of the medically relevant radionuclides require chelating chemistry for attachment to proteins or other targeting vehicles. Specific radionuclides from cyclotrons or reactors can be obtained in pure form for longer-lived isotopes, or as the products of generator elutions for short-lived isotopes.

Receptors are integral to all aspects of cellular function, such as proliferation, migration and communication, and triggering or blocking receptors with a mAb can induce either cell growth or death[42,43]. Preclinical experiments have demonstrated a reduced therapeutic effect against breast cancer and lymphoma, respectively, by rituximab and trastuzumab when the Fc receptor activation is absent[44,45]. Rituximab binding to CD20 seems to invoke a series of signalling events, including increased phosphorylation, phospholipase Cγ activation, c-Myc upregulation and induction of apoptosis in B lymphocytes[46–48]. Trastuzumab, which binds to HER2, has been postulated to have a direct antiproliferative signalling effect and blocks receptor–ligand interactions, causing downregulation of the receptor[49,50]. In addition, the mAb anti-Tac (daclizumab), which recognizes the 55-kDa α-chain of the interleukin-2 (IL-2) receptor (CD25), achieves therapy through interference of cellular signalling and functioning by blocking IL-2 binding, thereby inhibiting tumour-cell proliferation[51].

An mAb that modifies cell signalling might result in synergistic effects when used in conjunction with chemo- or conventional radiotherapy. Studies in animal models have served to confirm this, as anti-HER2 mAbs in combination with external beam radiation have resulted in antitumour effects in systems in which radiation or mAbs alone had minimal effect on tumour xenografts[52]. A number of ongoing clinical trials are investigating the effects of combined drug/targeted radiation therapy to integrate radioimmunotherapy into mainstream clinical cancer therapy. As an example, trastuzumab combined with paclitaxel or doxorubicin enhanced both the rates of response and the duration of response in patients with metastatic breast cancer[53].

Radioimmunoconjugates: clinical trials

Radioimmunotherapy has been evaluated in clinical trials across the full spectrum of malignancies[10,54]. Despite decades of research in the field, dissimilarities in experimental design and execution have complicated the direct comparison of the data obtained from separate sources. Comparisons are further complicated by a host of parameters, including dosing (protein and radioactivity), administrative methodology, radionuclide, targeting vehicle, and selection of both end point and the manner of its determination. Rather than an exhaustive coverage of the subject area, which is beyond the scope of this review, a selection of radiolabelled monoclonal antibodies in clinical development for the treatment of lymphohaematopioetic diseases are presented to shed light on the information gained. General reviews and those of a more specific nature, for example, on radiobiology, are available[10,54–60]. Considerable effort has been spent developing and evaluating peptides and mAb fragments and their variants, with respect to normal tissue toxicity and renal dose limitations in preclinical settings; these studies are also precluded from this review, as most of these studies are presently focused on imaging applications.

Most cytotoxic agents, whether in the form of chemo- or radiation therapy, have a low therapeutic index (benefit versus risk); that is, the maximal tumour-cell killing dose can also damage normal tissues. The use of mAbs as 'magic bullets' might enhance the therapeutic index because they selectively localize the cytotoxic agent. Most radioimmunotherapy clinical trials have used intact murine IgG mAbs. However, genetic engineering has allowed the development of chimeric and humanized (CDR-grafted) mAbs that are now being

evaluated in clinical trials[4]. Substantial effort continues in this arena with careful attention to rapid targeting with internalization, maximized retention through target antigen re-expression and minimized exposure of normal tissue. In a general sense, success has been limited in the treatment of solid tumours, whereas the greatest accomplishments have been made with lympho-haematopoietic malignancies, despite toxicity issues originating primarily from myelosuppression. The accessibility of these tumours and our ability to characterize tumour phenotypes, coupled with the ability to determine the stage of differentiation and their intrinsic radiosensitivities, also suggests that these cancers present themselves optimally for treatment. Antigenic heterogeneity and lack of tumour penetration of the antibody are not major impediments to radioimmunotherapy of lymphohaematopoietic disease, whereas these variables, coupled with myelosuppression and other dose-limiting toxicities (for example, hepatic, renal and gastrointestinal), have limited therapeutic doses from being actively delivered to solid tumours[3,4].

Ibritumomab tiuxetan (Zevalin; Biogen Idec) became the first therapeutic radiolabelled mAb to be approved by the FDA in February 2002. Zevalin is a murine anti-CD20 mAb labelled with ^{90}Y using an acyclic DTPA class BCA (tiuxetan) (FIG. 5), and is approved for the treatment of relapsed or refractory low-grade, follicular or transformed B-cell NHL. This indication includes patients with Rituxan (rituximab)-refractory follicular NHL. Zevalin has been approved as part of a therapeutic regimen involving Rituxan. Phase I/II studies with Zevalin demonstrated the antitumour efficacy and safety in patients with recurrent B-cell lymphoma, and also established 'pre-treating' patients with unlabelled mAb as part of the treatment protocol[61]. Targeted radiation therapy with doses up to 50 mCi resulted in minimal non-haematological toxicity and durable clinical responses. 'Pre-treatment' of patients decreased splenic uptake and urinary excretion, whereas it increased relative disease-site uptake. Both complete and partial responses (CRs and PRs) resulted from this approach, with a progression-free survival (PFS) of 72% 3–29+ months and a PFS of 78% with doses up to 40 mCi. Other trials have since established patient doses, and comparison to Rituximab (chimerized ibritumomab) immunotherapy indicated an improved overall response rate (ORR) (80% versus 56%) and a greater number of CRs (30% versus 16%). Furthermore, a 74% ORR was achieved with Zevalin in patients who failed Rituxan therapy. Interestingly, there has been a lack of correlation between dosimetric/pharmacokinetic parameters and haematological toxicity, indicating that toxicity might be inversely correlated with bone-marrow reserves in this patient population. When these various trials were integrated and analysed, a single dose of Zevalin was determined to be safe in patients with <25% bone-marrow involvement by NHL, adequate bone-marrow reserves, platelets >100,000 cells per μl and neutrophils >1,500 cells per μl[62].

A second anti-CD20 mAb regimen — tositumomab and ^{131}I tositumomab (Bexxar; GlaxoSmithKline) — received FDA approval in June 2003 for the treatment of patients with CD20-positive, follicular NHL, with and without transformation, whose disease is refractory to Rituxan and who have relapsed following chemotherapy. A Phase II multi-centre trial confirmed efficacy and safety in patients with relapsed or refractory low-grade or transformed low-grade NHL[63]. A study with Bexxar also demonstrated improved survival in patients with chemo-refractory NHL. As compared with 17% responding to chemotherapy, 65% of the Bexxar-treated patients experienced PRs or CRs averaging 6.5 months versus 3.4 months. The median response duration was ~eightfold longer for those receiving targeted radiation therapy versus chemotherapy only, with a corresponding CR of 20% and 3%, respectively[64]. A randomized study comparing tositumomab alone and with Bexxar has been presented with the goal of determining whether the ^{131}I provided any additional benefit to the patients. In the

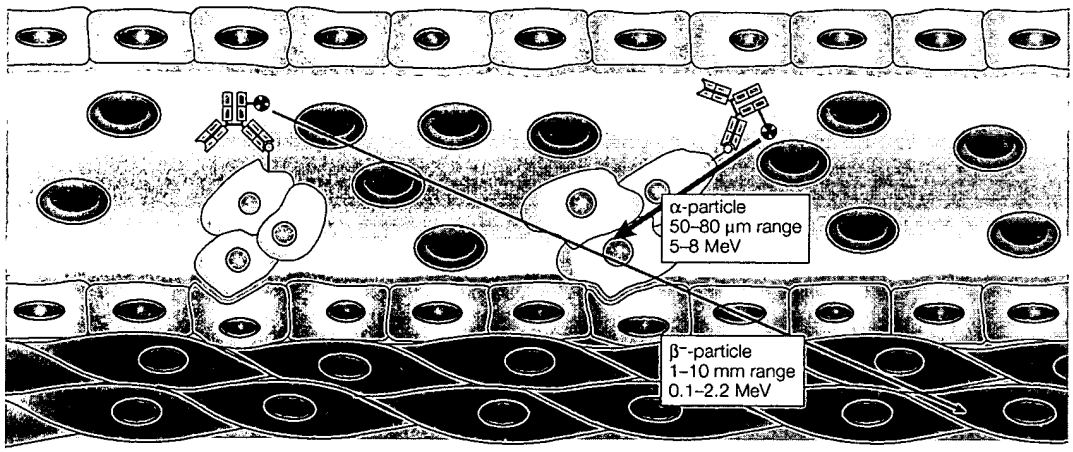

Figure 3 | **Comparison of path lengths and emission tracks of α- and β⁻-particle emissions used in antibody-targeted radiation therapy.** The β⁻-particle emissions occur in a spectrum of path lengths directly related to particle energy. The sparse energy track from these emissions is deposited over many cell diameters some distance from the decay event. The α-particle emissions occur at a discrete energy and path length, resulting in a high linear energy transfer. The dense energy track from these emissions is deposited directly from the decay event over only a few cell diameters, 50–80 μm in tissues.

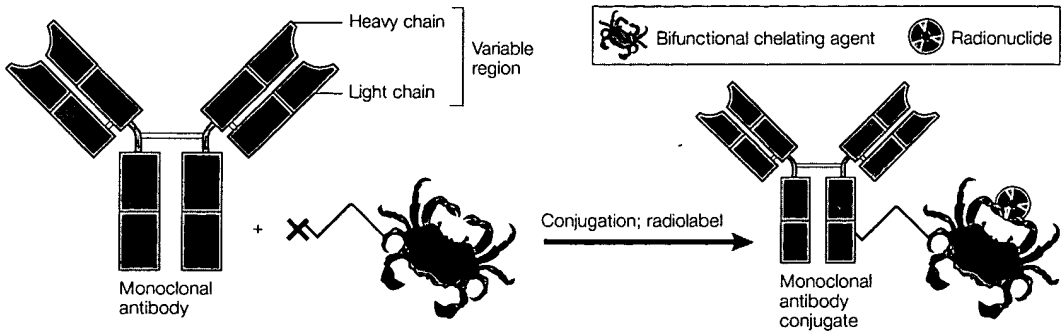

Figure 4 | **A general view of the conjugation of a bifunctional chelating agent to a monoclonal antibody.** Specifically, a bifunctional chelating agent possesses two functionalities. One portion binds (crab = chelos = chelate) metallic radionuclides, and the other portion bearing a reactive functional group (X) reacts and covalently binds to *N*-terminal and ε-amines from lysines on the antibody. In general, the metallic radionuclide is added last in this sequence, before purification of the final product; however, variations in which a pre-formed radiometal complex is conjugated to the monoclonal antibody are known.

words of the investigators, "all therapeutic outcome measures were significantly enhanced by the conjugation of ^{131}I to tositumomab."[65] In a follow-up study of 1,071 Bexxar-treated patients, no increased incidence of treatment-related myelodysplastic disease or acute myeloid leukaemia was found when this was the initial therapy, which might be an expected outcome of conventional chemotherapeutic and radiotherapeutic regimens[66].

The CR rate, remission duration and the ORR from either single or fractionated doses of B-cell malignancies has been more impressive in clinical trials administering high myeloablative doses in conjunction with either autologous bone-marrow transplantation (BMT) or stem-cell transplantation (SCT)[60,67–70]. This is significant, as it is often found that therapeutic doses of radioactivity delivered by an mAb cannot be administered without BMT or SCT support. In one such trial, patients with relapsed B-cell lymphoma were treated with ^{131}I-labelled anti-CD20 mAbs, including Bexxar[67]. Those patients developing myelosuppression underwent BMT. Complete remissions were noted, with several lasting up to 53 months. A later study with ablative Bexxar therapy and autologous SCT resulted in 86% ORs and 76% CRs. The PFS of these patients was 62% and the overall survival (OS) was 93%, with a median follow-up of two years[68]. Direct comparison between high-dose targeted radioimmunotherapy (HD-RIT) and conventional high-dose radiotherapy (HD-XRT) has also been evaluated. A PFS of 48% and 29% with an OS of 67% and 53% for HD-RIT and HD-XRT, respectively, was reported[69]. Disappointingly, most patients receiving HD-RIT eventually relapsed, leading to the proposal of including chemotherapy in the radioimmunotherapy regimen[70,71]. A recent report describes a Phase II trial in which CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine and prednisone) was followed by tositumomab/^{131}I-tositumomab for untreated follicular NHL[71]. Patients received CHOP therapy and, after four to eight weeks, a low dose of ^{131}I-tositumomab was administered for imaging and dosimetric calculations. Patients then received a therapeutic dose of ^{131}I-tositumomab. This treatment scheme resulted in 67% CRs and 23% PRs. The two-year PFS was estimated at 81% and the 2-year OS was 97%, with a median follow-up of 2.3 years. A Phase III clinical trial with CHOP in combination with Rituxan is ongoing. A treatment plan that combines chemotherapy, total body irradiation and radioimmunotherapy for the treatment of advanced acute lymphocytic leukaemia (ALL) and myelodysplastic syndrome (MDS) has also shown potential[72]. MDS patients treated with ^{131}I-labelled anti-CD45 mAbs had a median survival of 65 months (15–89 months), whereas the ALL patients had a disease-free survival (DFS) of 19, 54 and 66 months.

Issues pertaining to repeat treatments by either Bexxar or Zevalin potentially eliciting HAMA, although of concern owing to the murine nature of these mAbs, have been minimal. HAMA occurs <2% of the time with Zevalin, and there has been ~5–10% occurrence with Bexxar. Re-treatment has not been limited by this mechanism in immunocompetent patients.

Clinical trials at the University of California, Davis, USA, have focused on evaluating the therapeutic effectiveness of radioimmunotherapy using ^{67}Cu. Studies with ^{131}I-labelled Lym-1, a mAb with high affinity for the B chain of the HLA-DR10 antigen on malignant B lymphocytes, have demonstrated effectiveness in NHL patients. Patients who received up to 100 mCi per m^2 have responded to radioimmunotherapy[73]. A fractionated radioimmunotherapy study treated patients with ^{131}I-Lym-1 at four-week intervals. Remissions (71%) averaged 14 months in duration, with acceptable haematological toxicity[74]. A Phase I/II trial with ^{67}Cu-Lym-1 established the maximum tolerated dose (MTD) of a single dose at 50–60 mCi per m^2. In a dose-fractionation study, scintigraphy was used to determine dosimetry and pharmacokinetics[13]. Tumour regression was noted, with one CR lasting 12 months, although one patient required transfusions after five doses totalling 524 mCi. Good imaging, a high therapeutic index and favourable dosimetry with a ^{67}Cu-labelled mAb was achieved. The liver received the highest radiation absorbed dose; however, no hepatotoxicity was evident, which is an important point, as uncomplexed

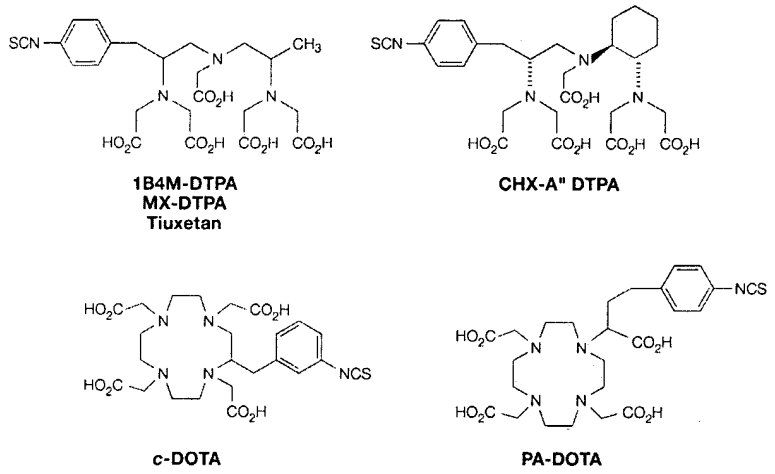

Figure 5 | **Selected examples of bifunctional chelating agents that have been or are currently in antibody-targeted radiation therapy clinical trials.** Conjugation occurs through the isothiocyanate of those agents depicted here. Metals/radiometals interact with the chelate via the amine and carboxylate groups.

Cu radionuclides localize in part to the liver[75]. A higher peak concentration in tumours, as well as a longer biological half-life, was achieved with ^{67}Cu compared with ^{131}I. The ^{67}Cu-labelled mAb delivered twice the mean radiation absorbed dose as ^{131}I, thereby demonstrating the importance of radiometal being retained within cells, rather than the de-halogenation and loss of effective dose found with ^{131}I (REF. 76).

This group has also evaluated Lym-1 radiolabelled with ^{90}Y. Therapy with ^{90}Y-Lym-1 (up to 0.37 GBq per m^2) resulted in disease stabilization and PR. High absorbed radiation dose to the liver was noted and led to the modification of the BCA. A catabolizable peptide linker was introduced for cleavage by cathepsin in hepatocytes, thereby facilitating excretion of the radiochelate during metabolic processing of the conjugate[77,78]. Clinical trials treating breast and prostate cancer using this linker as an element of the radioimmunoconjugate improved therapeutic indices. Tumour targeting was not significantly altered, no significant toxicities occurred and the absorbed radiation dose to the liver was reduced[77,79].

Adult T-cell leukaemia is an aggressive disease with a median survival of nine months. There is an overexpression of the IL-2 receptor by mature versus resting lymphocytes, thereby providing a selective target. A Phase I/II clinical trial was conducted to establish the dose and assess the efficacy of ^{90}Y-anti-Tac, which targets the IL-2 receptor[80]. Patients were treated with 5, 10 or 15 mCi, with an additional cohort treated at 10 mCi. Additional cycles of radioimmunotherapy (average of three) were administered as patients responded to therapy. Nine of the sixteen evaluable patients responded to the ^{90}Y-anti-Tac radioimmunotherapy (PR = 7, CR =2). At 20 months post-therapy, 35% of the patients were event-free and one patient remained in complete remission for more than three years. Six patients developed HAMA after one or two cycles precluding continued therapy. A Phase I/II trial with the humanized form of anti-Tac is ongoing at present.

Expression of CD33 on early myeloid progenitor cells and myeloid leukaemia cells provides yet another molecular target. The murine anti-CD33 mAb M195 has been shown to target leukaemia in patients, and a trial applying ^{131}I-M195 eliminated large tumour burdens[81]. Myelosuppression occurred at ≥135 mCi per m^2, allowing patients to undergo BMT. The effectiveness of the BMT was improved when ^{131}I-M195 (120–230 mCi per m^2) was combined with chemotherapy before a first or second BMT[81]. The data indicated that M195 could be effective in tumour reduction and that radioimmunotherapy had potential as part of a BMT regimen.

^{131}I-M195 has also been shown to be effective in the reduction of minimal residual disease. Patients in remission following retinoic-acid therapy who were positive for retinoic-acid receptor before ^{131}I-M195 therapy transiently converted to negative status following radioimmunotherapy. The median DFS was eight versus three months without radioimmunotherapy. Myelosuppression and the development of neutralizing HAMA permitted only a single dose of ^{131}I-M195 to be administered to patients, thereby reinforcing two significant obstacles to the further development of murine mAbs[81]. A humanized M195 (HuM195) has since been developed and found to be effective.

Myeloablative therapy with a ^{131}I-HuM195 requires several infusions to deliver sufficient radiation doses to the marrow. The half-life of ^{131}I impedes re-infusion of the stem cells and requires the patient to remain hospitalized in isolation. Subsequently, efficacy of HuM195 labelled with ^{90}Y was evaluated in a Phase I trial. Patients with relapsed or refractory acute myelocytic leukaemia were treated with ^{90}Y-HuM195. A reduction in bone-marrow blasts was achieved and some patients showed no evidence of disease for two to four weeks; one CR was noted. Conclusions indicated that ^{90}Y-HuM195 had potential as a component of a transplantation regimen[82].

Elimination of minimal residual disease in this patient population is viewed as the means of achieving long-term remission with prolonged DFS. To this end, ^{213}Bi-HuM195 has been administered to patients, marking the first antibody-targeted radiation therapy trial in humans using an α-emitter. Real-time imaging, necessitated by isotope half-life, was used to estimate pharmacokinetics and dosimetry[30]. The absorbed-dose ratio between the bone marrow, liver and spleen (sites of disease in leukaemia patients) and the whole body for the ^{213}Bi-labelled mAb was determined to be 1,000–10,000 times greater than ^{131}I and ^{90}Y (REF. 30). Patients had reductions in their peripheral blood leukaemia cells and a decrease in the percentage of bone-marrow blasts. Myelosuppression lasted 12–42 days (median of 22), with transient, low-grade liver function abnormalities seen in some patients. Patients were treated with up to 95 mCi in up to seven dose fractions with no toxicities evident[30].

Multi-step 'pre-targeting' protocols have been designed to disconnect the radionuclide from the targeting vector to improve the therapeutic index[83–85]. The goal

is to inject the mAb, allow the mAb sufficient time to target the tumour and to 'clear' remaining mAb from the circulation. One pre-targeting design exploits the affinity of biotin for streptavidin/avidin (SA) to maintain specificity. These protocols use a two- or three-step procedure to obtain adequate targeting, and then clearance, of circulating mAb. Clinical trials have been conducted in which the pre-targeting agent consisted of a mAb–biotin conjugate, while SA delivered the radionuclide[83]. The reverse design has also been evaluated in clinical trials. In this case, the pre-targeting moiety consisted of a mAb conjugated with SA, followed by treatment with radiolabelled biotin[84,85]. The SA–mAb localized at the tumour binds the radiolabelled biotin, whereas the unbound radiolabelled biotin is renally excreted, thereby enhancing tumour uptake versus background. The corollary to this strategy is that the vast majority of injected isotope is excreted, thereby requiring injection of large doses to counterbalance the clearance. Obviously, renal toxicity remains a concern when using this strategy. Using this latter motif, a Phase I/II trial was conducted that treated NHL patients with rituximab–SA conjugate injected sequentially, followed by a clearing agent (biotin-*N*-acetyl-glucosamine) and thereafter with 30 or 50 mCi per m^2 of ^{90}Y-DOTA-biotin[86]. Patients responded favourably, with only transient haematological toxicities. Using an appropriate mAb as the targeting vector, pre-targeting radioimmunotherapy protocols might have a place in the repertoire of treatment regimens for cancer patients. However, the greatest caveat remains the immunogenicity of the streptavidin component, which limits repeat therapies. This aspect, in part, might be obviated by application of the mAb fragment–streptavidin fusion protein tetramer; however, use of these engineered proteins remains to be fully evaluated[87]. One variation on pre-targeting has been the application of bispecific mAbs that bind to the cell and to a therapeutic element simultaneously[88]. One limitation to this methodology is the elimination of bi-valency in mAb binding, which potentially limits or lowers cell-binding affinity. However, eliminating immunogenic streptavidin makes this an attractive technology. The creation of bispecific mAbs that initially bind a radiometal complex possessing a reactive group that then covalently and irreversibly binds to the mAb is truly innovative, as it creates an 'infinite affinity' for the complex[89]. Evaluation in a clinical setting will provide a measure of the real value of this technology.

In summary, thirteen monoclonal antibodies have been approved by the FDA at present, and two of these are radiolabelled, both for the treatment of CD20$^+$ NHL: Bexxar, a murine IgG2a anti-CD20 mAb labelled with ^{131}I; and Zevalin, a murine IgG1 anti-CD20 mAb labelled with ^{90}Y via a BCA[61,68]. A 67% overall response rate has been achieved in NHL patients receiving Zevalin[61]. Experience with a single dose of Bexxar in 582 patients in five clinical trials has resulted in responses lasting from three months to five and a half years. A total of 161 patients (28%) had a CR with the median duration of the response lasting 4.8 years. The results with these two agents show the effectiveness of enhanced efficacy from the targeted radionuclide. The results also illustrate the rational approach of targeting a cell-surface antigen that is not shed or modulated, and which can also directly signal cell death through apoptosis, unlike other B-cell targets such as CD19 (REF. 46). This inherent activity of the target provides a true molecularly targeted combination therapy. This is a key factor to the success of both of these agents, and in particular for Bexxar, in that internalization and concomitant de-halogenation and excretion of the ^{131}I is minimized. Considerable efforts continue towards fully understanding the mechanisms of action in such instances, as well as in those regimens that combine various drugs (for example, paclitaxel and doxorubicin) and radiosensitizers (Gemzar)[71,90,91]. Although these might be acting in complete independence, some evidence exists — particularly with the anti-CD20 mAbs, trastuzumab and the anti-epidermal growth factor receptor antibody cetuximab (Erbitux; ImClone/Bristol-Myers Squibb) — that additive, synergistic or complementary modes of action might make cells more sensitive to radiation, thereby enhancing the therapeutic index[92].

Conclusion

After more than two decades, mAb-targeted therapies are generally recognized as making a significant impact on cancer therapy. The recent approvals of Zevalin and Bexxar have fuelled renewed enthusiasm for developing mAb-directed therapies. As such, their full potential is only now just beginning to be appreciated and understood.

However, despite the wealth of knowledge and capability in antibody engineering, the first two approved radiolabelled mAbs were murine in nature and subject to all of the resultant limitations; that is, immunogenicity and biological half-lives. Knowledge of actual clinical use of radiolabelled mAbs remains in its infancy in many respects, particularly with regard to therapies beyond lympho-haematological diseases, fractionated dosing and the rational construction of drug-combination cocktail therapies to functionally integrate targeted radiation therapy with established chemotherapies and external beam therapies. Clear evidence exists that value-added results are achieved by execution of these strategies.

Dominance of mAb therapies for the lympho-haematopoietic malignancies and their success in these cancers reflects their accessibility and radiosensitivity. Literature consensus seems to support mAb-based therapies of solid tumour applied in the treatment of minimal residual or micrometastatic disease and as a component of a multi-modality treatment regimen to provide the greatest benefit to patients. However, these limitations might have been arrived at through less than optimal targeting agents, sub-optimal chemistry, incorrect radionuclide choice and a less than rational experimental design, and so much of what has been achieved defines negative territory. There remains a continuing effort to refine and optimize all of the components to improve efficacy and minimize toxicity. The next decade should prove exciting as rational investigations applying the cumulative knowledge bring us towards making targeted radiation therapy a mainstream component for the treatment and management of cancer.

1. Kohler, G. & Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256**, 495–497 (1975).
2. Ehrlich, P., Herta, C. A. & Shigas, K. Ueber einige verwendungen der naphtochinosulfsaure. *Z. Physiol. Chem.* **61**, 379–392 (1904).
3. Schlom, J. in *Molecular Foundations of Oncology* (ed. Broder, S.) 95–134 (Williams and Wilkins, Baltimore, 1990).
4. Milenic, D. E. Radioimmunotherapy: designer molecules to potentiate effective therapy. *Semin. Radiat. Oncol.* **10**, 139–155 (2000).
5. Srivastava, S. & Dadachova, E. Recent advances in radionuclide therapy. *Semin. Nucl. Med.* **31**, 330–341 (2001).
6. Humm, J. L. Dosimetric aspects of radiolabeled antibodies for tumor therapy. *J. Nucl. Med.* **27**, 1490–1497 (1986).
7. O'Donoghue, J. A., Bardies, M. & Wheldon, T. E. Relationships between tumor size and curability for uniformly targeted therapy with β-emitting radionuclides. *J. Nucl. Med.* **36**, 1902–1909 (1995).
Defines the limitations and boundary conditions of appropriate particle emission energy matched to scale of tumour lesion for optimal energy deposition and therapy.
8. Mattes, M. J. *et al.* The advantage of residualizing radiolabels for targeting B-cell lymphomas with a radiolabeled anti-CD-22 monoclonal antibody. *Int. J. Cancer* **71**, 429–435 (1997).
9. Zhang, Y. *et al.* Comparative cellular catabolism and retention of astatine-, bismuth-, and lead-radiolabeled internalizing monoclonal antibody. *J. Nucl. Med.* **42**, 1538–1544 (2001).
10. Knox, S. J. & Meredith, R. F. Clinical radioimmunotherapy. *Semin. Radiat. Oncol.* **10**, 73–93 (2000).
A comprehensive review of targeted radiation therapy that presents the reader with the milestones and limitations that have been encountered as clinical trials have proceeded forward.
11. Mulligan, T. *et al.* Phase I study of intravenous Lu-labeled CC49 murine monoclonal antibody in patients with advanced adenocarcinoma. *Clin. Cancer Res.* **1**, 1447–1454 (1995).
12. Alvarez, R. D. *et al.* Intraperitoneal radioimmunotherapy of ovarian cancer with ^{177}Lu-CC49: a Phase I/II study. *Gynecol. Oncol.* **65**, 94–101 (1997).
13. O'Donnell, R. T. *et al.* A clinical trial of radioimmunotherapy with ^{67}Cu-2IT-BAT-Lym-1 for non-Hodgkin's lymphoma. *J. Nucl. Med.* **40**, 2014–2020 (1999).
14. McDevitt, M. R. *et al.* Radioimmunotherapy with α-emitting nuclides. *Eur. J. Nucl. Med.* **25**, 1341–1351 (1998).
15. Hassfjell, S. & Brechbiel, M. W. The development of the α-particle emitting radionuclides ^{212}Bi and ^{213}Bi for therapeutic applications. *Chem. Rev.* **101**, 2019–2036 (2001).
This review article provides an overview of the radiochemistry, chelation chemistry and preclinical studies that have led to the clinical use of ^{213}Bi.
16. McDevitt, M. R. *et al.* Tumor therapy with targeted atomic nanogenerators. *Science* **294**, 1537–1540 (2001).
17. Geerlings, M. W. Radionuclides for radioimmunotherapy: criteria for selection. *Int. J. Biol. Markers* **8**, 180–186 (1993).
18. Vaidyanathan, G. & Zalutsky, M. R. Targeted therapy using α-emitters. *Phys. Med. Biol.* **41**, 1915–1931 (1996).
19. Kurtzman, S. H. *et al.* ^{212}Bismuth linked to an antipancreatic carcinoma antibody: model for α-particle-emitter radioimmunotherapy. *J. Natl Cancer Inst.* **80**, 449–452 (1988).
20. Bethge, W. A. *et al.* Selective T-cell ablation with bismuth-213 labeled anti-TCRαβ as nonmyeloablative conditioning for allogeneic canine marrow transplantation. *Blood* **101**, 5068–5075 (2003).
21. Thorpe, P. E. & Derbyshire, E. J. Targeting of vasculature of solid tumors. *J. Control. Release* **48**, 277–288 (1997).
22. Hartmann, F. *et al.* Radioimmunotherapy of nude mice bearing a human IL2Rα-expressing lymphoma utilizing the α-emitting radionuclide-conjugated monoclonal antibody ^{212}Bi-anti-Tac. *Cancer Res.* **54**, 4362–4370 (1994).
23. Kassis, A. I., Harapanhalli, R. S. & Adelstein, S. J. Comparison of strand breaks in plasmid DNA after positional changes of auger electron-emitting iodine-125. *Rad. Res.* **151**, 167–176 (1999).
24. Makrigiorgos, G., Adelstein, S. J. & Kassis, A. I. Auger electron emitters: insights gained from *in vitro* experiments. *Rad. Environ. Biophys.* **29**, 75–91 (1990).
25. Michel, R. B., Brechbiel, M. W. & Mattes, M. J. A comparison of radionuclides conjugated to antibodies for single-cell kill. *J. Nucl. Med.* **44**, 632–640 (2003).
26. Packard, A. B., Kronauge, J. F. & Brechbiel, M. W. in *A Metalloradiopharmaceuticals II, Diagnosis and Therapy* (eds Clarke, M. J. & Sadler, P. J.) 45–116 (Springer, New York, 1999).
27. Ruegg, C. L. *et al.* Improved *in vivo* stability and tumor targeting of bismuth-labeled antibody. *Cancer Res.* **50**, 4221–4226 (1990).
28. Brechbiel, M. W. & Gansow, O. A. Synthesis of C-functionalized *trans*-cyclohexyldiethylene-triamine-pentaacetic acids for labelling of monoclonal antibodies with the bismuth-212 α-particle emitter. *J. Chem. Soc. Perkin Trans. I* 1173–1178 (1992).
29. Milenic, D. E. *et al. In vivo* evaluation of bismuth-labeled monoclonal antibody comparing DTPA-derived bifunctional chelates. *Cancer Biotherap. Radiopharm.* **16**, 133–146 (2001).
30. Jurcic, J. G. *et al.* Targeted α-particle immunotherapy for myeloid leukemia. *Blood* **100**, 1233–1239 (2002).
Describes the results of the first clinical trial using a monoclonal antibody targeted α-emitter, ^{213}Bi, for the treatment of leukaemia at the Memorial Sloan-Kettering Cancer Center.
31. Alvarez, R. D. *et al.* Intraperitoneal radioimmunotherapy of ovarian cancer with ^{177}Lu-CC49: a Phase I/II study. *Gynecol. Oncol.* **65**, 94–101 (1997).
32. Roselli, M. *et al. In vivo* comparison of CHX-DTPA ligand isomers in athymic mice bearing carcinoma xenografts. *Cancer Biotherap. Radiopharm.* **14**, 209–220 (1999).
33. Milenic, D. E. *et al. In vivo* comparison of macrocyclic and acyclic ligands for radiolabeling of monoclonal antibodies with ^{177}Lu for radioimmunotherapeutic applications. *Nucl. Med. Biol.* **29**, 431–442 (2002).
34. Novak-Hofer, I. & Schubiger, P. A. Copper-67 as a therapeutic nuclide for radioimmunotherapy. *Eur. J. Nucl. Med.* **29**, 821–830 (2002).
35. DeNardo, G. L. *et al.* Maximum tolerated dose of ^{67}Cu-2IT-BAT-LYM-1 for fractionated radioimmunotherapy of non-Hodgkin's lymphoma: a pilot study. *Anticancer Res.* **18**, 2779–2788 (1998).
36. Bass, L. A., Wang, M., Welch, M. J. & Anderson, C. J. *In vivo* transchelation of copper-64 from TETA-octreotide to superoxide dismutase in rat liver. *Bioconjugate Chem.* **11**, 527–532 (2000).
37. Rogers, B. E. *et al.* Comparison of four bifunctional chelates for radiolabeling monoclonal antibodies with copper radioisotopes: biodistribution and metabolism. *Bioconjugate Chem.* **7**, 511–522 (1996).
38. Wu, A. M. *et al.* High-resolution microPET imaging of carcinoembryonic antigen-positive xenografts by using a copper-64-labeled engineered antibody fragment. *Proc. Natl Acad. Sci. USA* **97**, 8495–8500 (2000).
39. Zalutsky, M. *et al.* Astatine-211 labeled human/mouse chimeric anti-tenascin monoclonal antibody via surgically created resection cavities for patients with recurrent glioma: Phase I study. *Neurooncol.* **4**, S103 (2002).
40. Milenic, D. E. Monoclonal antibody-based therapy strategies: providing options for the cancer patient. *Curr. Pharm. Design.* **8**, 1749–1764 (2002).
41. Cardarelli, P. M. *et al.* Binding of CD20 by anti-B1 antibody or $F(ab')_2$ is sufficient for induction of apoptosis in B-cell lines. *Cancer Immunol. Immunother.* **51**, 15–24 (2002).
42. Clynes, R. A., Towers, T. L., Presta, L. G. & Ravetch, J. V. Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. *Nature Med.* **6**, 443–446 (2000).
43. Johnson, P. & Glennie, M. The mechanisms of action of rituximab in the elimination of tumor cells. *Semin. Oncol.* **30**, 3–8 (2003).
44. Trauth, B. *et al.* Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science* **245**, 301–305 (1989).
45. Baselga, J. & Mendelsohn, J. Receptor blockade with monoclonal antibodies as anti-cancer therapy. *Pharmacol. Ther.* **64**, 127–154 (1984).
46. Cragg, M. S., French, R. R. & Glennie, M. J. Signaling antibodies in cancer therapy. *Curr. Opin. Immunol.* **11**, 541–547 (1999).
47. Deans, J. P. *et al.* Association of tyrosine and serine kinases with the B cell surface antigen CD20. Induction via CD20 of tyrosine phosphorylation and activation of phospholipase C-γ 1 and PLC phospholipase C-γ 2. *J. Immunol.* **151**, 4494–4504 (1993).
48. Maloney, D. G. *et al.* IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood* **90**, 2188–2195 (1997).
49. Sarup, J. C. *et al.* Characterization of an anti-p185HER2 monoclonal antibody that stimulates receptor function and inhibits tumor cell growth. *Growth Regulat.* **1**, 72–82 (1991).
50. Goldenberg, D. M. Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody, a novel agent for the treatment of metastatic breast cancer. *Clin. Ther.* **21**, 309–318 (1999).
51. Uchiyama, T., Nelson, D. L., Fleisher, T. A. & Waldmann, T. A. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. II. Expression of Tac antigen on activated cytotoxic killer T cells, suppressor cells, and on one of two types of helper T cells. *J. Immunol.* **126**, 1398–1340 (1981).
52. Pietras, R. J. *et al.* Monoclonal antibody to HER-2/neureceptor modulates repair of radiation-induced DNA damage and enhances radiosensitivity of human breast cancer cells overexpressing this oncogene. *Cancer Res.* **59**, 1347–1355 (1999).
53. Hortobagyi, G. N. & Perez, E. A. Integration of trastuzumab into adjuvant systemic therapy of breast cancer: ongoing and planned clinical trials. *Semin. Oncol.* **28**, 41–46 (2001).
54. Meredith, R. F., LoBuglio, A. F. & Spencer, E. B. Recent progress in radioimmunotherapy for cancer. *Oncology* **11**, 979–984 (1997).
55. Yu, J., Yin, D. & Wang, Y. Advances in monoclonal antibodies for radioimmunotherapy. *Hejishu* **25**, 393–400 (2002).
56. Hernandez, M. C. & Knox, S. J. Radiobiology of radioimmunotherapy with ^{90}Y ibritumomab tiuxetan (Zevalin) *Semin. Oncol.* **30**, 6–10 (2003).
Discusses the radiobiological effects of low-dose radiation, which ranges from induction of apoptosis to cell-cycle redistribution.
57. Hainsworth, J. D. Monoclonal antibody therapy in lymphoid malignancies. *The Oncologist* **5**, 376–384 (2000).
58. Mulford, D. A. & Jurcic, J. C. Antibody-based treatment of acute myeloid leukemia. *Expert Opin. Biol. Ther.* **4**, 95–105 (2004).
59. Waldmann, T. A. Immunotherapy: past, present and future. *Nature Med.* **9**, 269–277 (2003).
60. Pagel, J. M., Matthews, D. C., Applebaum, F. R., Berstein, I. D. & Press, O. W. The use of radioimmuno-conjugates in stem cell transplantation. *Bone Marrow Transplant.* **29**, 807–816 (2002).
Provides a recent overview of the application of the targeted radiation in the context of bone-marrow transplantation.
61. Knox, S. J. *et al.* Yttrium-90-labeled anti-CD20 monoclonal antibody therapy of recurrent B-cell lymphoma. *Clin. Cancer Res.* **2**, 457–470 (1996).
62. Witzig, T. E. *et al.* Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. *J. Clin. Oncol.* **20**, 2453–2463 (2002).
63. Vose, J. M. *et al.* Multicenter phase II study of iodine-131 tositumomab for chemotherapy-relapsed/refractory low-grade and transformed low-grade B-cell non-Hodgkin's lymphoma. *J. Clin. Oncol.* **18**, 1316–1323 (2000).
64. Kaminski, M. S. *et al.* Pivotal study of iodine I-131 tositumomab for chemotherapy-refractory low-grade or transformed low-grade B-cell non-Hodgkin's lymphomas. *J. Clin. Oncol.* **19**, 3918–3928 (2001).
65. Davis, T. *et al.* Long-term results of a randomized trial comparing tositumomab and iodine-131 Tositumomab (BEXXAR) with tositumomab alone in patients with relapsed or refractory low grade (LG) or transformed low grade (T-LG) non-Hodgkin's lymphoma (NHL). *Blood* **102**, A405–A406 (2003).
66. Bennett, J. M. *et al.* Assessment of treatment-related myelodysplastic syndromes (tMDS) and acute myeloid leukemia (tAML) in patients with low-grade non-Hodgkin's lymphoma (LG-NHL) treated with tositumomab and iodine-131 tositumomab (the BEXXAR therapeutic regimen). *Blood* **102**, A30 (2003).
67. Press, O. W. *et al.* Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. *N. Engl. J. Med.* **329**, 1219–1224 (1993).
68. Press, O. W. *et al.* Phase II trial of ^{131}I-B1 (anti-CD20) antibody therapy with autologous stem cell transplantation for relapsed B cell lymphomas. *Lancet* **346**, 336–340 (1995).
69. Gopal, A. K. *et al.* High-dose radioimmunotherapy versus conventional high-dose therapy and autologous hematopoietic stem cell transplantation for relapsed follicular non-Hodgkin lymphoma: a multivariable cohort analysis. *Blood* **102**, 2351–2357 (2003).
70. Press, O. W. *et al.* A phase I/II trial of iodine-131-tositumomab (anti-CD20), etoposide, cyclophosphamide, and autologous stem cell transplantation for relapsed B-cell lymphomas. *Blood* **96**, 2934–2942 (2000).
71. Press, O. W. *et al.* A phase 2 trial of CHOP chemotherapy followed by tositumomab/iodine I-131 tositumomab for previously untreated follicular non-Hodgkin lymphoma: Southwest Oncology Group Protocol S9911. *Blood* **102**, 1606–1612 (2003).
72. Matthews, D. C. *et al.* Phase I study of ^{131}I-anti-CD45 antibody plus cyclophosphamide and total body irradiation for advanced acute leukemia and myelodysplastic syndrome. *Blood* **94**, 1237–1247 (1999).

73. DeNardo, G. L. *et al.* Treatment of B-cell malignancies with I-131 Lym-1 monoclonal antibodies. *Int. J. Cancer* **3**, 96–101 (1988).
74. DeNardo, G. L. *et al.* Maximum tolerated dose, toxicity and efficacy of ^{131}I-LYM-1 antibody for fractionated radioimmunotherapy of non-Hodgkins lymphoma. *J. Clin. Oncol.* **16**, 3246–3256 (1998).
75. Durbin, P. W. Metabolic characteristics within a chemical family. *Health Phys.* **2**, 225–238 (1960).
76. DeNardo, G. L. *et al.* ^{67}Cu-versus ^{131}I-labeled Lym-1 antibody: comparative pharmacokinetics and dosimetry in patients with non-Hodgkin's lymphoma. *Clin. Cancer Res.* **5**, 533–541 (1999).
77. DeNardo, S. J. *et al.* Ytrrium-90/indium-111-DOTA-peptide-chimeric L6: pharmacokinetics, dosimetry and initial results in patients with incurable breast cancer. *Anticancer Res.* **17**, 1735–1744 (1997).
78. Li, M. & Meares, C. F. Synthesis, metal chelate stability studies, and enzyme digestion of a peptide-linked DOTA derivative and its corresponding radiolabeled immunoconjugates. *Bioconjugate Chem.* **4**, 275–283 (1993).
79. DeNardo, S. J. *et al.* Enhanced therapeutic index of radioimmunotherapy (RIT) in prostate cancer patients: comparison of radiation dosimetry for 1,4,7,10-tetraazacyclododecane-*N*, *N'*,*N'*,*N''*-tetraacetic acid (DOTA)-peptide *versus* 2IT-DOTA monoclonal antibody linkage for RIT. *Clin. Cancer Res.* **9**, S3938–S3944 (2003).
80. Waldmann, T. A. *et al.* Radioimmunotherapy of interleukin-2Rα-expressing adult T-cell leukemia with yttrium-90-labeled anti-Tac. *Blood* **86**, 4063–4075 (1995).
81. Jurcic, J. G. *et al.* Radiolabeled anti-CD33 monoclonal antibody for myeloid leukemias. *Clin. Cancer Res.* **55**, S5908–S5910 (1995).
82. Jurcic, J. G. *et al.* Potential for myeloablation with yttrium-90-HuM195 (anti-CD33) in myeloid leukemia. *Proc. Am. Soc. Clin. Oncol.* **19**, A7 (2000).
83. Paganelli, G. *et al.* Antibody-guided three-step therapy for high grade glioma with yttrium-90 biotin. *Eur. J. Nucl. Med.* **26**, 348–357 (1999).
84. Breitz, H. B. *et al.* Clinical optimization of pretargeted radioimmunotherapy with antibody-streptavidin conjugate and ^{90}Y-DOTA-biotin. *J. Nucl. Med.* **41**, 131–140 (2000).
85. Knox, S. J. *et al.* Phase II trial of yttrium-90-DOTA-biotin pretargeted by NR-LU-10 antibody/streptavidin in patients with metastatic colon cancer. *Clin. Cancer Res.* **6**, 406–414 (2000).
86. Weiden, P. L. *et al.* Pretargeted radioimmunotherapy (PRIT) for treatment of non-Hodgkin's lymphoma (NHL): initial phase I/II study results. *Cancer Biother. Radiopharm.* **15**, 15–29 (2000).
87. Forero, A. *et al.* Phase I trial of a novel anti-CD20 fusion protein in pretargeted radioimmunotherapy for B-cell non-Hodgkin's lymphoma. *Blood* (in the press).
88. Chatal, J.-F. *et al.* Bifunctional antibodies for radioimmunotherapy. *Hybridoma* **14**, 125–128 (1995).
89. Chmura, A. J., Orton, M. S. & Meares, C. F. Antibodies with infinite affinity *Proc. Natl Acad. Sci. USA* **15**, 8480–8484 (2001).

Describes an innovative approach to the delivery of chelated radiometals via a bispecific monoclonal antibody that binds and then covalently binds to the complex while binding to the cell surface.

90. Meredith, R. F. *et al.* Intraperitoneal radioimmuno-chemotherapy of ovarian cancer: a phase I study. *Cancer Biother. Radiopharm.* **16**, 305–315 (2001).
91. Cardillo, T. M., Blumenthal, R., Ying, Z. & Gold, D. V. Combined gemcitabine and radioimmunotherapy for the treatment of pancreatic cancer. *Int. J. Cancer* **97**, 386–392 (2002).
92. Harari, P. M. & Huang, S.-H. Head and neck cancer as a clinical model for molecular targeting of therapy: combining EGFR blockade with radiation. *Int. J. Radiat. Oncol. Biol. Phys.* **49**, 427–433 (2001).

Competing interests statement

The authors declare that they have no competing financial interests.

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